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Clinical Perspective on Use of Long-Read Sequencing in Prenatal Diagnosis of Thalassemia

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Guest: Dr. Diana Toledo is an Associate Lab Director at the Broad Institute's Clinical Research Sequencing Platform in Cambridge, MA.

Bob Barrett: This is a podcast from the journal *Clinical Chemistry*, a production of the American Association for Clinical Chemistry. I am Bob Barrett.

False positive or false negative results can have devastating consequences in all areas of laboratory medicine. But this is particularly true in prenatal diagnosis, when test results may be used to make decisions about a pregnancy for certain inherited conditions, currently available test methods work well and provide reliable actionable results. For others, the genetic sequence makes it difficult for routine test methods to accurately identify disease causing variants.

Alpha-thalassemia, a single gene disorder with high carrier frequency, is a perfect example as short read next-generation sequencing is unable to differentiate between the two alpha-globin genes. A research article and accompanying editorial in the March 2023 issue of *Clinical Chemistry* describes the application of long-read sequencing to the prenatal diagnosis of thalassemia. The editorial puts the research findings in context, specifically highlighting the performance characteristics of this new method and explaining how its use would change patient care decisions.

In this podcast, we are pleased to be joined by the editorial's lead author. Dr. Diana Toledo is an Associate Lab Director at the Broad Institute's Clinical Research Sequencing Platform in Cambridge, Massachusetts. She has a Master's degree in genetic counseling and a PhD in genetics, with subsequent experience in clinical molecular pathology and diagnostics.

So, Dr. Toledo, your editorial explains the clinical utility of long-read sequencing and the prenatal diagnosis of thalassemia. So what is long-read sequencing and how is it better than the standard testing methods?

Diana Toledo: So, standard testing methods used in genetic diagnosis of alpha-thalassemia and beta-thalassemia are basically PCR-based approaches, and they require a known target. So you really have to know what you're targeting or probing when you build your assay and before you run your first test. And

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this can be a limitation of PCR-based approaches. Sequencing methods allow for more variation in the DNA sequence to be uncovered. However, most sequencing methods that are clinically used today are something known as short-read sequencing. This is not ideal for regions of the genome that are highly repetitive or homologous. So the alpha-globin and beta-globin genes that are associated with thalassemia, these are very homologous genes to each other; they're pseudogenes.

So this new technology is utilizing a long-read sequencing approach, which is able to accurately sequence these regions of the genome that do have high homology, thus making it a better choice and technology for assaying genes like these genes and others like it. It also provides slightly higher specificity so, a better true negative rate, and also meaning a lower false positive rate. And in the cohort that we based this editorial off of, 15 pregnancies had additional or corrected variant findings, accounting for an increase in yield of almost 8% in that cohort when using the long-read sequencing method over the PCR-based methods.

Bob Barrett: Well, that certainly sounds like an improvement, but this increase in specificity, or decrease in false positive rate, seems incremental. Why implement something new for such small gains in diagnostic performance?

Diana Toledo: So, the stakes are quite high during prenatal testing. So at least one of the pregnancy decisions that came from the original testing using those PCR-based methods may have actually changed due to a false positive that was resulted at that time. So this pregnancy was terminated based on false positive results. But after testing with this new method, though retrospectively, this fetus was found to be a carrier for beta-thal and likely would not have been affected with the disease.

So this type of scenario really kind of exemplifies why small increases in positive predictive value of a test really has very high level of clinical utility in the prenatal setting.

Bob Barrett: Sounds like a lifesaving change there, okay. Can you explain some differences between genetic testing in the prenatal setting compared to the post-natal setting and also describe some of the special considerations?

Diana Toledo: Yeah, so prenatal genetic screening and testing has been a routine part of maternal-fetal medicine for some time. It started with chromosomal aneuploidy, or abnormality screening, in those with either ultrasound anomalies or maybe advanced maternal age. And now this field has really evolved to include diagnosis of many other genetic disorders during the prenatal period. So there's a very tight timeline

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when it comes to prenatal diagnosis. Between the time of a sample actually being collected, to results being returned back to the patient, and then a decision potentially having to be made about that pregnancy.

This all has to be done by a certain sort of deadline and which we know in the US it looks very different state by state, let alone across the world. There's a lot of pressures for those who are involved in this process, from the patient and her pregnancy, to the clinicians and scheduling visits, and ultrasounds and scheduling these testing procedures, to even the clinical labs and our turnaround times and what we provide back to the patient. And another caveat really with prenatal genetic screening and testing is that we can't see our patient. So the patient being the fetus, we can't see the patient or have other sort of clinical evidence surrounding a potential diagnosis. The only thing we're able to see is really the ultrasound and if there's nothing specific on the ultrasound, then there's no other piece of clinical evidence to go along with the genetic testing results. So you really have to be very sure that the false positive rate is very, very low for a test and that that positive predictive value of your assay is very high.

Bob Barrett: Well, since nothing is perfect, does this new long-read sequencing approach have any limitations?

Diana Toledo: Yes, so there are some cons definitely to long-read sequencing. This method has a higher level of complexity than its PCR-based counterparts. The instrumentation involved in long-read sequencing is also much more costly to run and costly to obtain. And then those who run it also need to be, I would say, more specialized. The protocols are also more detailed and labor-intensive and things like that. So this new long-read sequencing method, it also has a longer turnaround time in the lab, so the assay takes longer to run than the standard PCR-based methods. PCR methods take approximately three days for at least a rapid prenatal case like these cases, while the long-read method takes closer to eight days before results are available. And as I just mentioned, those days during the prenatal period are very, very crucial. So a good compromise, although might be costly, would be to sort of run both of these in parallel and provide preliminary results after the PCR-based tests are done, but then final results, potentially, once the long-read sequencing is done.

Bob Barrett: Well, finally, Dr. Toledo, are there any other takeaway points or recommendations you'd like to leave us with?

Diana Toledo: Yeah, so ultimately, I think any method that reduces the risk of false positive findings in the prenatal period, no matter how incremental, has great clinical utility. At the large scale, I

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think this helps build the public's trust in genetic testing, especially in this critical prenatal period.

Bob Barrett: That was Dr. Diana Toledo from the Broad Institute's Clinical Research Sequencing Platform in Cambridge, Massachusetts. She was the lead author of an editorial describing the clinical impact of long-read sequencing for the prenatal diagnosis of thalassemia. Her editorial was published in the March 2023 issue of *Clinical Chemistry*, and we're happy she has been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.

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